

Amendment and Response Under 37 CFR §1.116

Page 2 of 12

Serial No.: 09/884,894

Filed: June 19, 2001

Title: BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY AND METHODS OF USE

### Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

- 1-8. Canceled
9. (Withdrawn) A method for inhibiting the replication of a microbe in the gastrointestinal tract of an animal, comprising administering to an animal a *Bifidobacterium* that secretes a siderophore, and measuring the presence of a microbe that was present in the gastrointestinal tract of the animal prior to administration, where a decrease in the presence of the microbe in the animal after administration of the *Bifidobacterium* indicates inhibition of the replication of the microbe.
10. (Withdrawn) The method of claim 9 further comprising growing the *Bifidobacterium* under iron limited conditions before administration.
11. (Withdrawn) The method of claim 10 wherein growing the *Bifidobacterium* under iron limited conditions comprises growth in the presence of an iron chelator.
12. (Withdrawn) The method of claim 9 wherein the animal is a human.
13. (Withdrawn) The method of claim 9 wherein the microbe is a prokaryotic microbe.
14. (Withdrawn) The method of claim 13 wherein the prokaryotic microbe is selected from the group consisting of *E. coli*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Clostridium difficile*, and *Clostridium perfringens*.
15. (Withdrawn) The method of claim 9 wherein the gastrointestinal tract is the large intestine.

**Amendment and Response Under 37 CFR §1.116**

Page 3 of 12

Serial No.: 09/884,894

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16-19. Canceled

20. (Withdrawn) A method for establishing a Bifidobacterium flora in the gastrointestinal tract of an animal comprising administering to an animal a Bifidobacterium that secretes a siderophore, and measuring the presence of the Bifidobacterium in the gastrointestinal tract of the animal after administration.

21. (Withdrawn) The method of claim 20 further comprising growing the Bifidobacterium under iron limited conditions before administration.

22. (Withdrawn) The method of claim 20 wherein the gastrointestinal tract is the large intestine.

23. (Withdrawn) The method of claim 20 wherein the animal is a human.

24. (Withdrawn) The method of claim 23 wherein the human is an infant selected from the group consisting of an immature infant, a premature infant, and a mature infant.

25. (Withdrawn) The method of claim 23 wherein the administration occurs after the human has undergone antibiotic therapy.

26. (Withdrawn) The method of claim 23 wherein the administration occurs after the human has undergone chemotherapy.

27. (Withdrawn) A method for preventing the replication of microbes in a food, the method comprising adding to the food a Bifidobacterium that secretes siderophore.

28. (Withdrawn) A method for decreasing the risk of colon cancer comprising administering to an animal a *Bifidobacterium* that secretes a siderophore, and detecting the presence of aberrant

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Page 4 of 12

Serial No.: 09/884,894

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crypt foci in the colon of the animal, where a lower number of aberrant crypt foci compared to an animal not administered the *Bifidobacterium* indicates a decrease in the risk of colon cancer.

29. (Currently amended) A composition comprising [[a]] an isolated *Bifidobacterium* that secretes a siderophore that inhibits growth of a *Lactococcus lactis*, wherein the composition comprises substantially no free iron, comprises an iron chelator, or a combination thereof.

30. (Withdrawn) A method for obtaining a secreted siderophore from a *Bifidobacterium*, the method comprising incubating a *Bifidobacterium* under iron limited conditions, and isolating the siderophore.

31. (Withdrawn) A method for preparing a siderophore, the method comprising incubating a *Bifidobacterium* under iron limited conditions, and sterilizing the culture.

32. (Withdrawn) The method of claim 31 wherein the culture is sterilized by removing essentially all water from the culture.

33. (Withdrawn) A sterile composition comprising a siderophore obtained from a *Bifidobacterium*.

34. (Withdrawn) An isolated siderophore obtained from a *Bifidobacterium*.

35. (Withdrawn) An isolated siderophore that binds Fe<sup>2+</sup>, the siderophore obtained from a *Bifidobacterium*.

36. (Withdrawn) A method for decreasing an amount of free iron in a composition, the method comprising adding to a composition a secreted siderophore obtained from a *Bifidobacterium*.

Amendment and Response Under 37 CFR §1.116

Page 5 of 12

Serial No.: 09/884,894

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37. (Withdrawn) A method for decreasing an amount of free iron in a composition, the method comprising adding to a composition an isolated siderophore obtained from a *Bifidobacterium*.
38. (Withdrawn) A method for inhibiting the replication of a microbe in a composition, the method comprising adding to a composition a secreted siderophore obtained from a *Bifidobacterium*.
39. (Withdrawn) A method for inhibiting the replication of a microbe in a composition, the method comprising adding to a composition an isolated siderophore obtained from a *Bifidobacterium*.
40. (Withdrawn) A method for altering the expression of a siderophore in a *Bifidobacterium*, the method comprising incubating under iron limited conditions a *Bifidobacterium* that does not secrete a siderophore, and selecting for a *Bifidobacterium* that replicates in the iron limited condition.
41. (Currently amended) A composition consisting essentially of [[a]] an isolated *Bifidobacterium* that secretes a siderophore that inhibits growth of a *Lactococcus lactis*.
42. (Withdrawn) A sterile composition consisting essentially of a siderophore obtained from a *Bifidobacterium*.
43. (Currently amended) A composition comprising [[a]] an isolated *Bifidobacterium* that can secrete a siderophore that inhibits growth of a *Lactococcus lactis*, wherein the composition is for use as a probiotic.
44. (Currently amended) A composition comprising [[a]] an isolated *Bifidobacterium* that can secrete a siderophore that inhibits growth a *Lactococcus lactis*, and a food.